Letters to the Editor

Gamma irradiated blood samples: unsuitability for haemostatic evaluation of high risk patients

Over the past 40 years, several "new" viruses have been discovered which cause haemorrhagic and often fatal disease in man; Lassa, Ebola, and Marburg are but three examples. The haemostatic defect(s) in these diseases remain(s) largely undefined, partly because blood samples from infected patients can only be handled by centres equipped with P-4 containment facilities.¹ To determine the feasibility of using inactivated viral specimens for haemostatic analysis we studied the effects of gamma irradiation on several laboratory coagulation variables.

For plasma assays, one unit of time-expired, fresh frozen plasma was thawed at 37° C, and 0.5 ml portions were aliquoted into sets of 1.8 ml cryule tubes, six tubes to each set. For serum assays, 10 ml of fresh blood from a healthy donor were placed in a glass tube in a water bath at 37° C; after one hour the middle portion of the serum was divided equally into a further set of six cryule tubes. Three tubes from each set were exposed to 4×10^6 rads of gamma radiation using a gamma cell containing ⁶⁰Co (model 220; Atomic Energy of Canada Ltd, Ottawa, Canada) and three were left unirradiated; during the procedure, all test and control samples were kept at below 4° C in dry ice. Specimens were stored at -70° C until analysed.

We measured prothrombin times (PT), partial thromboplastin times (PTTK), and thrombin times (TT) manually by standard techniques; fibrinogen concentrations by the Clauss method; factor VIII by a standard one-stage assay; von Willebrand factor antigen (vWFAg) by an ELISA method modified from that of Short *et al*; ristocetin cofactor (RiCof) by platelet aggregometry using washed platelets²; antithrombin III

(ATIII) by a microtitre amidolytic assay modified from that of Odegaard *et al*³; protein C by a microtitre amidolytic (snake venom activated) assay⁴ using British Standard (NIBSC, Hertfordshire); protein S by an ELISA technique using polyclonal antiserum (Dakopatts, Buckinghamshire) and rabbit anti-human peroxidase conjugate (concentrations were standardised using pooled plasma from 20 donors); and serum thromboxane B₂ (TXB₂) by radioimmunoassay (Amersham, Buckinghamshire).

With the exception of vWFAg, all these tests were considerably affected by gamma irradiation (table). Thrombin times, PTS, and PTTKs were all prolonged in irradiated samples compared with control samples, and there was generally considerable variability in the extent of prolongation in replicate aliquots. Concentrations of fibrinogen, factor VIII, RiCof, antithrombin III and protein C, which were all obtained by functional assays, showed varying degrees of reduction after gamma irradiation. Concentrations of serum TXB₂ and protein S, although determined by immunological methods, were similarly affected.

These data show that gamma irradiation at a dose sufficient to inactivate class IV viral agents⁵ profoundly affects most haemostatic variables in serum and plasma. No method of viral inactivation has yet been described which does not affect the concentrations of coagulation factors in human blood. Samples obtained from patients suspected of harbouring a class IV pathogen must therefore be analysed in a maximum containment laboratory.

D CUMMINS

T SOUTHEE*

KAREN WALSH

IJ MACKIE

SJ MACHIN

Department of Haematology,
Middlesex Hospital,
Riding House Street, London W1

Table Effect of gamma radiation on laboratory coagulation assays

Laboratory test	Irradiated	Non-irradiated
PT (s)	20, 25, 52	18, 20, 21
PTTK (s)	51, 56, 135	38, 41, 38
Thrombin time (s)	20, 19, 19	13, 12, 12
Fibrinogen (g/l)	<0.10, 0.84, 0.50	2.10, 2.20, 2.20
Factor VIII (IU/ml)	0.14, 0.24, 0.11	0.48, 0.46, 0.50
vWFAg (IU/ml)	0.50, 0.48, 0.56	0.48, 0.52, 0.52
RiCof (IU/ml)	<0.05, <0.05, 0.11	0.72, 0.72, 0.72
ATIII (IU/ml)	0.27, 0.52, 0.37	0.94, 0.95, 1.08
TXB ₂ (ng/ml)	180, 85, 264	327, 314, 330
Protein C (IU/ml)	0.12, 0.21, 0.16	0.76, 0.78, 0.80
Protein S (%)	32, 11, 72	88, 89, 86

References

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Idiopathic haemochromatosis in north Portugal: association with haplotype A3B7

The association of certain HLA antigens and haplotypes with idiopathic haemochromatosis is well documented, and their pattern of distribution has been defined in several countries. The antigen most strongly associated with idiopathic haemochromatosis is HLA-A3 and the haplotypes more frequently linked to the disease are A3B7 and A3B14.¹⁻³

In 1986 we started a systematic search for cases of idiopathic haemochromatosis in the north of Portugal that has led to the identification of 30 patients to date. Preliminary studies of the first unrelated patients and families⁴ indicated an association of antigen A3 and haplotype A3B7 with the disease in this region.

Seventeen unrelated, HLA typed, patients with idiopathic haemochromatosis (15 men and two women), aged between 21 and 69 years were included in this study. Haemochromatosis was diagnosed according to previously established clinical, biochemical, and histopathological criteria.⁵

One hundred and eighteen family members (55 men and 63 women) of 10 unrelated subjects were studied. All subjects were HLA typed; evaluation of their iron state was carried out by routine measurements of serum iron, total iron binding capacity, transferrin saturation and serum ferritin. In